2021 Center for Produce Safety Symposium
Session 1 – Forum on *Cyclospora cayetanensis*

Executive Summary

The 12th Annual Center for Produce Safety (CPS) Research Symposium kicked-off on June 15, 2021, with a virtual forum on *Cyclospora cayetanensis*, a parasite and human pathogen of growing priority for the fresh produce industry. The session featured presentations from leading researchers covering the known biology of the organism, surveillance efforts, history of produce related outbreaks, current detection challenges and opportunities for infection prevention. The agenda for the program can be found here and a recording of the entire session can be found here. CPS has funded and continues to fund research focused on *Cyclospora* detection survival in fresh produce growing environments and control. A *Cyclospora* resource page with links to these research programs can be found on the CPS website.

1. ***Cyclospora cayetanensis has a complex biological life cycle that presents both challenges for study and opportunities for control.*** There are 21 species of *Cyclospora,* but humans are the only known host for *Cyclospora cayetanensis*. The parasite is excreted from humans as unsporulated oocysts in fecal material and requires a 7–15-day cycle outside the human body, i.e., in the environment, to become sporulated oocysts which if ingested by humans through contaminated food or water, may then cause illness. It is not known what drives sporulation in the environment but rainfall or moisture, temperature, and exposure to sunlight are thought to be important. It continues to be difficult to study the biology of *Cyclospora* because it is difficult to isolate enough oocysts to experiment with. Much is yet to be learned about the importance and biochemical control of the sporulation process, oocyst survivability in the environment and mechanisms to reduce survivability or interrupt sporulation and modes of transmission.
2. ***Cyclospora is no longer considered just a "travel-related illness"***. Cyclosporiasis was historically thought of as an illness acquired through drinking "bad" water when traveling to a tropical area. In the late 1990's, *Cyclospora* outbreaks in North America began to be linked to produce starting with raspberries (1996) followed by basil, mesclun salad and fruit salad, and extending to sugar snap peas, cilantro, vegetable trays, salad mix, and red cabbage in the 2000's. Many of these were imported produce but by 2018 and continuing into 2020, ingredients of packaged salads grown in the U.S. were also linked to cyclosporiasis outbreaks.
3. ***There is a "cyclosporiasis season"***. *Cyclospora* infections tend to be seasonal with the peak being between May 1st and August 31st. It is not known what factors drive seasonality. CDC and public health departments conduct surveillance all year long but conduct "enhanced surveillance" during the "cyclosporiasis season" specifically focusing on patient travel and fresh produce consumption histories among victims. It is important to note that during cyclosporiasis season there are often multiple outbreaks presumably from different sources occurring simultaneously, but we lack the scientific ability to genetically distinguish them.
4. ***New technologies are providing opportunities to more effectively detect Cyclospora***. Historically the only way to detect *Cyclospora* was through microscopy. Since the organism is generally present in low numbers and preparing and viewing samples is cumbersome, detection was inefficient. More recently dead-end ultrafiltration (DEUF) using large volumes (50-liters) of water has been shown to

be a more effective way to concentrate oocysts to ease detection of environmental water [Mattioli 2019]. An initial DNA-based detection method, FDA BAM 19a was plagued with false positives owing to the large number of species genetically related to *C. cayetanensis* and false negatives due to interference from chemicals in various food matrices. The multi-laboratory validated FDA BAM 19b qPCR method incorporates more effective sample preparation and better selectivity and specificity down to as little as 5-10 oocysts and has replaced BAM 19a as the preferred detection tool for *Cyclospora* in fresh produce. As BAM 19b becomes more readily practiced by researchers, government and commercial laboratories and the genetics of Cyclospora are elucidated, further improvements are likely to emerge. The investigation and quantification of the 2018 cyclosporiasis outbreak tracked to vegetable trays and the 2020 outbreak associated with bagged salad were greatly aided by the use of BAM 19b. *Even with improved methods, product testing in raw or finished products has little value owing to the very low levels found in foods; the ultimate example of searching for a very small needle in a large haystack*. FDA BAM 19c is the preferred method for analyzing high volume water samples for *Cyclospora*. The whole genome sequencing approaches that precisely identify specific bacterial pathogen strains are currently not possible with *Cyclospora* because it's biology and genetics are more complex.

5. ***Transference of Cyclospora to fresh produce is through human feces, either directly or indirectly***. Surface irrigation waters contaminated with human feces can act as a conduit for *Cyclospora* transmission to the crop. Human feces contamination of harvest tools or equipment used postharvest may also play a role. Since *Cyclospora* can be found in irrigation water applied to fields, workers boots, and gloves may also serve to further spread oocysts as they move around the production environment. Similarly, fecal contamination from animals that commonly consume human feces may also spread *Cyclospora.* Flooding events that overwhelm municipal sewage systems or ineffective sewage treatment strategies may also cause transference of oocysts to the environment and ultimately onto a crop. *The most effective way to control Cyclospora is to control human fecal contamination of production environments*.
6. ***A caution: Cyclospora can be found in open water sources***. Studies of irrigation ponds in the Southeastern U.S. revealed that 28-percent of irrigation ponds tested positive for *Cyclospora* and other human fecal indicators [Mattioli 2019]. A three-year surveillance of irrigation canals in Arizona [Lopez 2019] yielded 7% Cyclospora positives. While the prevalence of *Cyclospora* seems low, it is important for growers who are dependent on the use of these irrigation canals or ponds to *prioritize tracking the source of their water to determine if there are potential risks for human fecal contamination and assess the risk of product contamination*. It is important to note that positive PCR-based tests do not mean the oocysts are in the proper infective stage or even alive; just that the DNA is there. The same Arizona/Rio Grande study examined wastewater using quantitative PCR and found that 46% of Rio Grande Valley and 55% of Arizona raw sewage (influent) samples were positive for a *C. cayetanensis*-specific gene sequence while 26% in the Rio Grande and 37% of Arizona treated sewage or effluent samples were positive for the same sequence. Again, while these data reflect the presence of *Cyclospora* DNA and not necessarily live oocysts, it does serve as a caution for users of open irrigation water sources to closely evaluate their systems to determine if

there is a risk it could be compromised by leakage from sewage treatment operations. The detection of *C. cayetanensis*-specific gene sequences in sewage effluents also indicates that *Cyclospora* may be present in the surrounding human population suggesting the *importance of handwashing for employees contacting the products and proper maintenance and sanitation of portable toilets and avoidance of spillage in field locations*.

7. **Inactivation methods for *Cyclospora are currently unavailable for produce, but when developed, must be implementable by growers or packers***. *Cyclospora* oocysts are resistant to chlorine sanitizers, fungicides and pesticides employed in the produce industry. Microwave and thermal energy have been shown to be effective, but these treatments are not likely compatible with maintaining fresh produce quality or practical with current product handling, washing and packing practices. Ongoing research is exploring treatments with gamma irradiation, ozone and UV [Lenaghan 2020]. CPS has funded additional research projects examining the use of filtration and zero valent iron and sand filtration to remove or eliminate *Cyclospora* [Kniel 2019].

***Acknowledgements**: The Center for Produce Safety would like to thank session 1 presenters and moderators for their work and dedication to produce safety. More detail on the research projects can be found at www.centerforproducesafety.org. This executive summary is meant to inform and provoke thought with an eye towards inspiring readers to examine their own produce safety programs and to use the research to make improvements. It is not meant as a directive on what must be done to produce safe food. If you have additional questions, please feel free to contact Bonnie Fernandez-Fenaroli (info@centerforproducesafety.org). Thank you.*